Short Communications

Effect of ethanol and acetone on photochemical reactions in isolated chloroplasts of *Phaseolus vulgaris*

O.T. de Villiers* and H.J. Marais
Department of Agronomy and Pastures, University of Stellenbosch, Stellenbosch, 7600 Republic of South Africa

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The effect of ethanol and acetone on $^{14}CO_2$ fixation, oxygen evolution and electron transport were studied in isolated chloroplasts of *Phaseolus vulgaris*. Ethanol up to a concentration of 1% and acetone up to a concentration of 0,5% had little effect on $^{14}CO_2$ fixation whereas higher concentrations of both solvents inhibited this process severely. In the case of oxygen evolution and electron transport, concentrations of both solvents up to 5% had little or no inhibitory effect, whereas a concentration of 10% showed a marked inhibitory effect.

Die invloed van etanol en asetoon op $^{14}CO_2$-fiksering, suurstofvrystelling en elektronoordrag in geïsoleerde chloroplaste van *Phaseolus vulgaris* is nagegaan. Etanol tot 'n konsentrasie van 1% en asetoon tot 'n konsentrasie van 0,5% het geen invloed op $^{14}CO_2$-fiksering gehad nie terwyl hoër konsentrasies van beide oplosmiddels die proses sterk geïnhibeer het. In die geval van suurstofvrystelling en elektronoordrag het konsentrasies tot 5% van beide oplosmiddels geen of min invloed gehad terwyl 'n konsentrasie van 10% die prosesse sterk geïnhibeer het.

Keywords: Acetone, chloroplasts, ethanol, *Phaseolus vulgaris*, photochemical reactions

*To whom correspondence should be addressed

The low water solubility of most herbicides and plant hormones creates a practical problem when these compounds are used to study their effects on photochemical reactions in isolated chloroplasts. It is therefore often necessary to first dissolve such compounds in organic solvents such as ethanol or acetone, before they are added to a chloroplast suspension. To ensure that the observed results are due to the added compound, it is therefore necessary to determine the effect of the solvents as such on the various reactions. The following experiments were thus carried out to determine the effects of ethanol and acetone separately on certain photochemical reactions in isolated chloroplasts.

Beans (*Phaseolus vulgaris* L., var. Top Crop) were grown in moist Perlite at room temperature (23° ± 2°C). Light with an intensity of 9,6 Wm^{-2} at the level of the primary leaves was supplied by a combination of fluorescent and incandescent lights; a 16-h light and 8-h dark regime was employed. Isolation of chloroplasts and determination of the different photochemical reactions were done as previously described (De Villiers & Koch 1985).

In experiments in which the effect of ethanol or acetone was assayed, the solvent was added to chloroplasts in Erlenmeyer flasks to a concentration (v/v) of 0,5%, 1%, 5% or 10% in the final volume (De Villiers & Koch 1985). Chlorophyll content of the chloroplasts was determined by the method of Arnon (1949). All experiments were repeated three times, and the significant differences were assessed by means of an analysis of variance according to Snedecor & Cochran (1967).

Ethanol up to 1% concentration had little effect on $^{14}CO_2$ fixation, whereas acetone at the same concentration inhibited this process by 20% after 15 min (Table 1). At the higher concentrations, however, both ethanol and acetone exhibited a marked inhibitory effect. After 15 min the inhibition by 5% and 10% ethanol was 81% and 87% respectively. Acetone, at the same concentrations, inhibited this process by 84% and 95% respectively after 15 min. Similar results on the effect of these solvents on different biochemical processes were obtained in enzymatically isolated single cells from leaves and hypocotyl tissue of *Phaseolus vulgaris* (De Villiers *et al.* 1977; De Villiers *et al.* 1980). In the latter case both ethanol and acetone at concentrations up to 1%, had little inhibitory effect on CO_2 fixation, RNA synthesis, protein synthesis and lipid synthesis. At concentrations of 3% and higher both solvents severely inhibited these processes in the isolated cells.

Concentrations up to 5% of both ethanol and acetone had little or no inhibitory effect on oxygen evolution or electron transport (Table 1). At higher concentrations, however, both ethanol and acetone inhibited both reactions. Ethanol at a concentration of 10% inhibited oxygen evolution and electron transport by 36% and 61% respectively. Acetone, at the 10% level, inhibited oxygen evolution completely whereas electron transport was inhibited by 76%.

The above data indicate that both ethanol and acetone have a similar effect on photochemical reactions in chloroplasts. The severe inhibition induced by the higher concentrations is possibly the result of their effect on membrane permeability, thereby leading to disruption of the chloroplasts. Another possibility is that essential components of the electron chain are soluble in these solvents and are thus removed from the chloroplasts (Goodwin 1966). A microscopic examination of cells treated with 1% and 2% acetone showed that membrane abnormalities and cytoplasmic degeneration were more

Table 1 The effect of ethanol and acetone on different photochemical reactions in chloroplasts isolated from leaves of *Phaseolus vulgaris*

Solvent	Concentration %	$^{14}CO_2$ fixation[a]	Oxygen evolution[b]	Electron transport[c]
		% inhibition		
Ethanol	0,5	0	0	0
	1,0	4	1	3
	5,0	81*	3	4
	10,0	87*	36*	61*
Acetone	0,5	8	0	0
	1,0	20*	4	2
	5,0	84*	6	5
	10,0	95*	100*	76*

* Means differ significantly *LSD* (P = 0,05) from the control (no solvent present)
[a]Rate of $^{14}CO_2$ fixation of control = 301,1 dpm mg^{-1} chl × 10^4
[b]Rate of oxygen evolution of control = 1188,7 µl O_2 mg^{-1} chl h^{-1}
[c]Rate of electron transport of control = 99,0 µmol ferricyanide reduced mg^{-1} chl h^{-1}

prevalent than in normal cells (Davis *et al.* 1978).

The results of this study could thus serve as a guide to the solvent concentrations that may be used in studying photochemical reactions in isolated chloroplasts of *Phaseolus vulgaris* without any detrimental effect of the solvents on the reactions.

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The seedfly *Ophiomyia lantanae* and other factors responsible for reducing germination in *Lantana camara* forms found in Natal

J.L. Graaff

Plant Protection Research Institute, Private Bag X134, Pretoria, 0001 Republic of South Africa

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The effect of infection with the seedfly *Ophiomyia lantanae* Frogg. on the germination of seeds from different forms of *Lantana camara* L. was investigated. Results indicate that infection does reduce germination, in some cases quite significantly. However, it seems that the seedfly is not the only cause of low germination; seed dormancy, non-viable seeds and seeds without embryos are also possible causes of the low germination percentages often obtained from non-infected seeds.

Die effek van besmetting deur die saadvlieë, *Ophiomyia lantanae* Frogg. op die ontkieming van sade van verskillende *Lantana camara* L. vorms is ondersoek. Resultate het aangedui dat die besmetting deur die vlieë wel in sommige gevalle die ontkieming van die sade betekenisvol verminder het. Dit blyk tog dat die saadvlieg nie die enigste faktor is wat bydra tot die verminderde ontkieming nie. Saad-rus, onvrugbare sade en sade sonder embrio's is ook moontlike redes vir die lae ontkiemingspersentasies wat dikwels verkry word by sade wat nie deur die vlieg besmet is nie.

Keywords: Biological control, germination, *Lantana camara, ophiomyia lantanae*

Lantana camara L. originiates from South America and has become established as a weed in many areas of South Africa (Stirton 1978). The *L. camara* found in South Africa is not a homogeneous species, there are at least 40 distinct local forms (Stirton 1978). Spies & Stirton (1982) describe the species as a polypoid with chromosome numbers ranging from 2n to 6n. This wide variety of forms has lead to a number of problems in the field of biological control. For example, most of the established biocontrol agents, *Teleonemia scrupulosa, Octonoma scabripennis* and *Uroplata girardi* (Cilliers 1982), will only attack the leaves of certain preferred forms, thus leaving the plants with less palatable leaves to continue growing (Van de Venter 1982). Another insect, a seedfly *Ophiomya lantanae* Frogg. oviposits in the unripe berries of *L. camara*, apparently irrespective of the form. The usefulness of the seedfly as a biological control agent is uncertain as it is not known whether or not the laying of eggs within the berry has any adverse effect on the seed's subsequent ability to germinate. The seedfly is already widely established in South Africa and it would appear that its presence does reduce the chances of finding fully developed seed embryos (Cilliers 1982), but is seems that no one has been able to relate infection with the seedfly to any specific effect on germination.

Reproduction in *L. camara* can take place in a number of ways; roots will sucker if damaged (anonymous 1982) and stem cuttings also root easily. However, seeds are the most common method of reproduction. In South Africa, *L. camara* flowers prolifically and produces seeds all year round. In India the seed production of *L. camara* is so great that the seeds were tested and successfully used as a viable source of animal